ASSESSMENT OF THE RELATIONSHIP BETWEEN IRON STATUS, DIETARY INTAKE, PERFORMANCE, AND MOOD STATE OF FEMALE ARMY OFFICERS IN A BASIC TRAINING POPULATION

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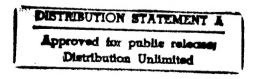
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DISCLAIMERS

The views, opinions, and/or findings in this report are those of the authors, and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other official documentation.

Human subjects participated in this study after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRDC Regulation 70-25 on the use of volunteers in research.

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LIST OF ACRONYMS & ABBREVIATIONS

AMEDD Army Medical Department

ANOVA analysis of variance

APFT Army Physical Fitness Test

CAN Computerized Analysis of Nutrients

CSFII Continuing Survey of Food Intake by Individuals

DEP depleted

DWHRP Defense Women's Health Research Program

ET endurance time to exhaustion

Hb hemoglobin HR heart rate

IUDs intrauterine devices

MMIL monthly menstrual iron loss

MRDA Military Recommended Dietary Allowances

NHANES National Health & Nutrition Examination Survey

NOR normal

OBC officer basic training cycle

PMBL menstrual blood loss per period

POMS Profile of Mood States

RBC red blood cells

RDA Recommended Dietary Allowances

TIBC total iron binding capacity

USAMRMC U.S. Army Medical Research & Materiel

Command

USARIEM U.S. Army Research Institute of Environmental

Medicine

VO₂max maximal oxygen uptake

VE minute ventilation

VO₂peak peak oxygen uptake

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This report is part of a comprehensive study supported by the 1994 Defense Women's Health Research Program (DWHRP, Grant # W4168021) that compared nutritional, iron, and health status of officers and enlisted women entering Army basic training. Additional results from this study will be presented in subsequent reports.

EXECUTIVE SUMMARY

This study was undertaken to determine the relationship between iron status, nutrition, physical and cognitive performance, and mood state of women during an 8-week officer basic training course at Fort Sam Houston, TX during the summer class of 1995. Volunteers were recruited from a class of 75 women. Fifty seven volunteers completed evaluation during Week 1; forty five women were evaluated during Week 8. Questionnaires were utilized to acquire demographic data, as well as nutritional intake and exercise habits prior to entry on active duty for training. Biochemical measures of iron and nutritional status as well as assessment of aerobic and cognitive performance, and mood state were determined at Week 1 and Week 8. Nutritional intake during training was obtained at Week 7 by diet records for seven consecutive days.

Measures of iron status upon entry to active duty revealed that, while the mean ferritin value was within normal range, 33% of the women were iron deficient based on serum ferritin levels ($<20 \mu g/l$), and 7% were anemic based on low hemoglobin levels (<12 g/dl). By the completion of basic training, iron deficiency and anemia were found in 64% and 13% of the women, respectively.

Nutrient intakes prior to active duty were assessed by food frequency questionnaire. Mean intakes met or exceeded the Recommended Dietary Allowances (RDA) for all nutrients except energy, iron, and folic acid (Food and Nutrition Board, National Research Council, 1989). Reported nutrient intakes during basic training, as assessed by 7-day diet records, were compared to the Military Recommended Dietary Allowances (MRDA), which reflect increased nutrient needs of military personnel (U.S. Department of the Army, AR 40-25, 1985). Mean intakes were low for energy, protein, folic acid, magnesium, and zinc. Dietary iron intake was less than the allowance in 50% of the women. However, serum markers of iron status were not significantly related to dietary iron intakes.

The physical fitness of the subjects entering basic training was well above average for comparably-aged servicewomen and improved significantly during the eight weeks of training. There was no effect of iron depletion, either assessed cross-

sectionally during the pre-training period or longitudinally as a consequence of the eight weeks of basic training, on any measure of physical performance as measured by maximal treadmill testing and the Army Physical Fitness Test (APFT).

No relationship was seen between iron status and mood states or reaction-time. A significant increase in subjective fatigue post-basic training was seen. Other mood scales followed the same pattern of increased distress, although the changes were not statistically significant.

Basic training had a negative effect on iron and nutritional status in these women, but there was no demonstrable effect of iron depletion on selected performance measures. Although this study examined the effects of acute iron deficiency on performance, it did not assess the impact of chronic iron deficiency as women continue to serve in their military specialties. Future research should examine the effects of chronic iron deficiency on women to assess its health and military performance effects and investigate nutritional interventions that might prevent such deficiencies.

INTRODUCTION

Iron deficiency is one of the most prevalent nutritional problems, occurring more commonly in women than men. It has been divided into three stages depending on severity: iron depletion, with decreased serum ferritin levels reflecting loss of iron stores; iron deficient erythropoiesis depicted by a decrease in transferrin saturation; and finally, a significant decrease in circulating hemoglobin, indicating iron deficiency anemia (Table 1) (Finch and Huebers, 1982; Gillen et al., 1991; Prasad and Prasad, 1991; Massey, 1992; Lyle et al., 1992; Haymes, 1993). In the United States, approximately 25% of adolescent girls and 20% of women ages 18-45 have been found to be iron depleted; 14% of adolescent girls and 9.6% of women have been classified as having iron-deficient erythropoiesis; and 5% of adolescent girls and women have been found to be anemic (Cook et al., 1976; Dallman et al., 1984; Expert Scientific Working Group, 1985; Looker et al., 1997).

Table 1. Stages of Iron Deficiency

Parameter	Normal ± SD	Iron Depletion	Iron-Deficient Erythropoiesis	Iron Deficiency Anemia
Transferrin IBC (µg/dl)	330 ± 30	360	390	410
Ferritin (µg/l)	100 ± 60	<20	<10	<10
lron (µg/dl)	115 ± 50	115	<60	<40
Transferrin saturation (%)	35 ± 15	30	<16	<16
RBC protoporphyrin (µg/dl RBC)	30	30	100	200
Hemoglobin (g/dl)	>12	>12	>12	<12

Serum ferritin is considered the most sensitive measure of iron status, because of the ability to distinguish between true iron deficiency and the anemia of chronic infection (Cook and Finch, 1979). Levels below 20 μ g/l are associated with the absence of iron in the bone marrow levels; levels between 12 and 20 μ g/l are referred to as pre-latent iron deficiency (Haymes, 1993).

Decreased performance during physical exercise by women with impaired iron status has been reported in numerous investigations in the United States, as well as in other countries (Tumbi and Dodd, 1990; Edgerton et al., 1979; Gardner et al., 1977; Magazanik et al., 1991). Impaired iron status has been found in some groups of women engaged in moderate levels of aerobic exercise. Any iron deficiency induced by exercise training would be compounded by heavy menstrual flow or increased blood volume associated with training (Haymes, 1993; Lyle et al., 1992; Telford et al., 1993; Clement and Sawchuck, 1984). Prevalence of iron depletion in women athletes has been reported to range from 25% to 35%. However, most studies have reported that the prevalence of iron deficiency anemia in athletes did not exceed that normally found in U.S. women (Balaban et al., 1989; Colt and Heyman, 1984; Deuster et al., 1986; Haymes and Spillman, 1989; Nickerson et al., 1989; Clement et al., 1987; Haymes et al., 1986; Risser et al., 1988).

As women in this country become increasingly more involved in sports and fitness programs, it is important to assess fitness levels of those women currently entering the military. As of 31 July 1995, women comprised 13.2% of the U.S. Army active duty personnel (68,970) (Department of Defense, 1995). Although women have become an integral part of the Army, adequate information is lacking on their iron and nutritional status and how that status may affect physical and cognitive performance. As women in the military enter more job specialties that are physically demanding or require endurance, they must be medically and physically capable of performing their duties. If iron status remains compromised from the initiation of Army training, and these women are not evaluated and treated for iron deficiency, physical performance may be negatively affected throughout their careers. Impaired performance or endurance could result in injury to the individual female soldier or other members of the unit. Since the Army is attempting to meet requirements to increase work capability with fewer soldiers, loss of personnel due to injury or illness can have a major impact

on the mission. The nutritional status and physical performance of male soldiers has been studied extensively, but little has been reported for females, especially officers (Bugge et al., 1979; Jones et al., 1992; Knapik et al., 1994; Moore et al., 1992; Patton et al., 1980; Vogel et al., 1986; Westphal et al., 1995).

In a recent study of female Army soldiers attending enlisted basic training, a significant percentage (56%) of the women were iron deficient (Westphal et al., 1995). Iron status became progressively worse in a majority of the soldiers evaluated, suggesting a possible deleterious effect of training on iron stores. Significant differences in physical fitness scores were seen between those women who were iron depleted and women with normal iron status, but the relationships between various stages of iron deficiency and physical performance were not evaluated. Although iron deficiency was demonstrated in many female soldiers, the mechanisms involved in the apparent deterioration of iron status have not been identified in this population group.

The difficulty in maintaining iron balance may be explained by the body's limited iron absorption and excretion. The body is genetically designed to absorb heme iron (found in animal tissue foods), which may have been appropriate for past generations when humans were believed to be predominantly hunters. Our diet has changed progressively to include more grain products which have a much lower availability of iron.

Various health organizations recommend increasing dietary fiber for its protective factor against several disease states. These recommendations are directed at a healthy, adult population consuming a typical Western diet, such as the female population in this study. Although dietary iron intake may appear adequate upon initial evaluation, further study reveals that a majority of the iron being consumed is from grain products which provide less available iron (Finch and Huebers, 1982).

The most recent nutrition survey in the U.S. (Third National Health and Nutrition Examination Survey, NHANES III) (Interagency Board, 1995) has reported an average iron consumption for women of 10.5 mg iron/day, and for teenage girls 10.8 mg iron/day, which is well below the Recommended Dietary Allowance (RDA, 1989) of 15 mg/day. Surveys of women athletes have reported a higher iron intake of 12-18

mg/day (Risser et al., 1988; Clement and Asmundson, 1982; Snyder et al., 1989).

Analysis of the diet of women in the Westphal et al. (1995) study revealed a dietary intake of iron suboptimal when compared to the MRDA of 18 mg/day (U.S. Department of the Army, 1985). Other nutrients playing a role in iron status, such as folate and vitamin C, were also lower than the MRDA. Over two-thirds of the women stated they had been trying to lose weight prior to active duty, which could have an impact on nutritional intake. Dietary factors not evaluated (which may have affected serum iron values) included fiber, phosphate, and meat protein intake (Haymes, 1993; Cook and Finch, 1979; Finch and Huebers, 1982; Telford et al., 1993; Clement and Sawchuck, 1984). Besides diet, other variables such as smoking, menstrual flow, and increased plasma volume from aerobic exercise have also been shown to affect serum iron values (Leggett et al., 1990; Gillen et al., 1991; Senay, 1975).

Iron status of enlisted women entering the Army has been assessed previously (Westphal et al., 1995); however, limited data are available on female officers as they enter the military. Westphal et al., failed to show a clear relationship between physical performance and iron deficiency; however, an association between anemia and poor Army Physical Fitness Test (APFT) performance was observed.

Although there has been considerable research examining the effects of iron status on physical performance, little attention has been paid to cognitive performance or mood states. Psychological monitoring of the effects of overtraining and its relation to psychological staleness has been done using the Profile of Mood States (POMS) questionnaire (Morgan et al., 1987a). Examination of mood profiles of female swimmers and distance runners has shown that negative psychological mood changes are associated with overtraining and decreases in physical performance (Morgan et al., 1987b; O'Connor et al., 1991). This mood-overtraining relationship is similar to that found in male athletes (Morgan et al., 1987b; Morgan et al., 1988; O'Connor et al., 1991). Because iron status is also affected by overtraining (Clement and Sawchuck, 1984), it may be hypothesized that moods would also be affected by changes in iron status.

Since mood states can directly predict certain aspects of performance (Bugge, et

al., 1979), they provide a simple, non-invasive means to quantify the effects of various nutritional treatments on certain aspects of military performance. In elite athletes, scores on mood state profiles correlate with performance (Morgan and Johnson, 1978). However, other studies on athletes (Risser et al., 1988) and non-athletes have shown no effect of iron deficiency on symptoms and mood (Beutler et al., 1960). No other data on the relationship of iron status to mood state are, to the best of our knowledge, available. Many studies have documented changes in mood associated with administration of specific food components (Lieberman et al., 1986), but little information is available on the relationship of iron status to mental state. Anecdotal reports suggest that moods like "fatigue" and "vigor" may be related to iron status, but this has not been documented.

With identification of those factors having an impact on iron status in female soldiers, programs for screening, treatment, and prevention can be developed to improve iron status in female soldiers. If an individual can be returned to her full physical potential, she will be able to maximally contribute to successful completion of the military mission.

The primary objectives of this study were 1) to identify variables associated with decreased iron status in female soldiers, 2) to evaluate the association between iron status and physical and cognitive performance, and 3) to assess the effect of officer basic training on female soldiers' iron status.

METHODS

SAMPLE POPULATION

Volunteers from one officer basic training cycle (OBC) at the Army Medical Department (AMEDD) Center and School, Fort Sam Houston, Texas participated in this study. Their military job specialties included nursing, dietetics, social work, hospital administration, veterinary medicine, and occupational therapy. Complete demographic characteristics are available in Appendix A. Fifty-seven women (from a class of 75) between the ages of 18-25 years were included; 12 were subsequently dropped due to pregnancy, illness, or training injury. Differences in subject numbers in tables reflect incomplete or missing data. Volunteers were briefed on the purpose and potential hazards of the study, and read and signed a volunteer agreement form before being allowed to participate. Investigators adhered to AR 70-25 and USAMRMC Regulation 70-25 on Use of Volunteers in Research. Data were collected at the beginning (Week 1) and completion (Week 8) of OBC.

BASIC TRAINING SCHEDULE

All students attended the core training program for 8 weeks, and spent a majority of the time in classroom training during a full eight-hour duty day. One major field exercise was scheduled during Week 6 of training: students participated in field training for 3 days and nights at Camp Bullis, TX. Students had evenings, nights, and weekends free for studying, personal business, or recreation.

BACKGROUND HISTORY

Demographic data were collected with self-administered questionnaires that included questions on age, education, geographic location, marital status, and ethnic background. Sections were also included that inquired about smoking history, reproductive health, use of vitamin and mineral supplements, and consumption of beverages containing tannates and phosphates. Responses to menstrual history questions were used to estimate menstrual blood loss per period (MBLP) and monthly

menstrual iron loss (MMIL); calculations were made using procedures reported by Pate et al. (1993). Subjects reported frequency and length of menstrual periods as well as number of tampons or napkins used per day and the degree to which the tampons or napkins were saturated (on a scale of light=1, medium=2, heavy=3) on each day of menstrual flow. MBLP was calculated as the product of the duration of menstrual flow (in days), the number of tampons or napkins used per day, and their mean saturation on each day of flow. The MMIL was calculated by MBLP x hematocrit. Median blood loss is 30.0 ml, which, with a mean menstrual interval of 28 days, will translate to a loss of 0.5 mg of iron a day throughout the complete menstrual cycle (Hallberg et al., 1966; Hallberg and Rossander-Hultén, 1991).

PHYSICAL ACTIVITY PRIOR TO ACTIVE DUTY

Energy expenditure from leisure-time physical activity was determined from calculations of activities reported on a modification of the Minnesota Leisure Activities Questionnaire (Taylor et al., 1978; Jones et al., 1992), with intensity codes based on mean body weight, utilized for each activity. The formula used to determine weekly energy expenditure for each activity was as follows: activity intensity code x number of days/week for activity x minutes per episode. Weekly energy expenditures (kcal/activity/week) for each subject's listed activities were then added and the total was divided by 7 to determine individual kcal/day energy expenditure. A listing of reported leisure activities and their intensity codes can be found in Appendix B.

FOOD FREQUENCY QUESTIONNAIRE

A food frequency questionnaire was administered to all study participants at Week 1 to assess eating patterns and food preferences prior to active duty. The food frequency data were collected using a modified version of the Health Habits and Diet Questionnaire (Block and Wys, 1987). The brief, 60-item version evaluates 18 major nutrients and includes foods representing approximately 93% of total United States caloric consumption. This version produces estimates for a wide range of nutrients with validity and reproducibility similar to that of the full-length questionnaire used in numerous previous food consumption surveys (Block et al., 1990). Nutrient estimates for dietary assessment are based on the NHANES II nutrient content database

(Smucker et al., 1989). Foods are grouped into six categories by food types: fruits and vegetables; entrees; breads, salty snacks, spreads; breakfast foods; sweets; and dairy foods and beverages.

DIET RECORD

Because officers attending OBC are not required to eat meals in a dining facility, participants were asked to keep a record of all foods and beverages they consumed for seven days during Week 7 of basic training. Forms were filled out by each subject and were reviewed daily by a trained dietary data collector.

NUTRITIONAL ANALYSIS

Dietary intake was analyzed for the following nutrients: energy, protein, fat (total, saturated, and cholesterol), carbohydrate, vitamin C, thiamin, riboflavin, niacin, vitamin B₆, folic acid, vitamin B₁₂, vitamin A, calcium, phosphorus, magnesium, iron, zinc, potassium, and sodium. Analysis was completed using the Computerized Analysis of Nutrients (CAN) System (Rose et al., 1989) developed at USARIEM. The nutrient database primarily used was the Nutrient Data Base for Individual Food Intake Surveys, which was adapted for the Continuing Survey of Food Intake by Individuals (CSFII)(U.S. Department of Agriculture, Release 7, 1991).

Nutrient database food codes were assigned to foods based on descriptive and nutrition information provided from the CSFII. In some cases, recipes were coded if no appropriate match was found in the database. Nutrient values from food labels and manufacturers' data were added to the study database for those items not found in the existing database.

Menu items were categorized into major and minor food groups (Appendix C). The relative iron contribution of each of these major food groups (dairy, meats, grains, legumes, vegetables, fruits, desserts, fats and oils, and others) was determined.

BLOOD CHEMISTRY

Blood samples were obtained at two points during the study (Week 1 and Week 8) to assess iron and nutritional status. The day prior to each collection, individuals were reminded that no food or fluid (except plain water) was permitted after 2100 hours on each of the evenings preceding the blood draws. Three separate 15 ml samples were collected into vacutainers: one 15 ml blood sample was taken to provide serum, and two 15 ml blood samples (EDTA and heparin as anticoagulants) were taken to provide unclotted whole blood, plasma, and erythrocytes. Following collection, blood samples were refrigerated at 6-10° C until processing. Serum (or plasma) and cells were separated and the different fractions frozen (-20°C) until shipment to the clinical laboratory at Pennington Biomedical Research Center. Samples were drawn on each participant for analysis of serum iron, ferritin, transferrin concentration, transferrin saturation, iron-binding capacity, hematocrit, and hemoglobin to determine iron status of each individual. Serum haptoglobin concentration was measured to determine whether accelerated intravascular hemolysis resulted from physical training. Red cell folate, serum vitamin B₁₂, vitamin C, retinol binding protein (RBP), and plasma magnesium were also analyzed using the same samples.

Iron and total iron binding capacity were analyzed using the ferrozine method on the Beckman Synchron CX7 (Beckman Instruments, La Brea, CA); magnesium was measured using the calmagite method. Hematocrit, hemoglobin, and red blood cell count were analyzed using a Coulter STKS hematology analyzer (Coulter Corporation, Miami, FL). Ferritin was measured using a chemiluminescent method on a Sanofi Pasteur ACCESS immunochemistry analyzer (Sanofi Diagnostics Pasteur, Inc., Chaska, MN). Haptoglobin and RBP were measured using rate nephelometry on the Beckman Array, an automated protein analyzer (Beckman Instruments, La Brea, CA). All tests were verified daily with standard quality control procedures including calibration if necessary and running multiple levels of quality control material with each run of analyses. Standardization has been confirmed by participation in the College of American Pathologists Survey.

Vitamin status was measured by direct assay or by biochemical indices summarized in Table 2. Vitamin B₁₂ and folate were analyzed using chemiluminescent

methods on a Sanifi Pasteur ACCESS immunochemistry analyzer; red blood cell folate was also analyzed on the ACCESS. Whole blood was processed with lysing reagent at the time of collection which both lyses the red blood cells and stabilizes folate within the sample. Ascorbic acid was measured by colorimetric assay using an ascorbate oxidase method on the Beckman Synchron CX7. These methods have been reported in more detail in Moore et al. (1992).

Table 2. Biochemical indicators used to assess vitamin and mineral status.

Vitamin/Mineral	Biochemical Indicators
Vitamin B ₁₂ (cobalamin)	Serum cobalamin concentration
Folacin	Erythrocyte folate Serum folate
Vitamin C (ascorbic acid)	Serum ascorbic acid concentration
Magnesium	Serum total magnesium
Iron	Serum total iron Serum total iron binding capacity (TIBC) Serum ferritin

MOOD STATES

Mood states associated with training and iron status were assessed using the POMS questionnaire. The POMS was administered via laptop computers and was assessed just prior to the treadmill test at the beginning and completion of OBC. The POMS assesses subjective mood states via a 65-item survey. Each item is rated on a five-point scale from feelings of "not at all" to "extremely." The response set of "how you are feeling right now" was used. The POMS assesses six mood scales: tension, depression, anger, vigor, fatigue, and confusion (McNair et al., 1971). The POMS total mood disturbance score was calculated as: Vigor - (Tension + Depression + Anger +

Fatigue + Confusion) + 100. The value of 100 is added to eliminate negative mood scores as has been done previously (Morgan et al., 1988).

FOUR-CHOICE REACTION TIME

The four-choice visual reaction time test was administered immediately after the POMS and prior to the treadmill test at the beginning and completion of OBC. This test was also administered on laptop computers as has been reported in previous studies (Banderet and Lieberman, 1989). Two-hundred fifty visual stimuli were presented at one of four different spatial locations on the screen. The participant had to indicate the correct spatial location of each stimulus by striking one of four adjacent keys on the computer keyboard. Measurements included correct hits, latency of correct hits, incorrect hits, latency of incorrect hits, premature errors (responding before the presentation of the stimulus) and time-out errors (response latency greater than one second). Performance on choice reaction time has been shown to be sensitive to a wide number of stressors (Bonnet, 1989; Dinges, 1992; Banderet and Lieberman, 1989).

ANTHROPOMETRY

Height, body mass, and body circumference measurements were taken during the first and eighth week of training. Height was measured to the nearest 0.5 cm with an anthropometer, while mass was measured to the nearest 0.1 kg on a calibrated scale (SECA). For both measurements, subjects were barefoot and dressed in T-shirt and shorts. Body fat was estimated using the standard Army circumference equations in accordance with AR 600-9, The Army Weight Control Program (U.S. Department of the Army, 1994). Circumference measurements of the neck, wrist, forearm, and hips were made in duplicate with a fiberglass tape measure.

PHYSICAL ACTIVITY DURING BASIC TRAINING

Physical training during the course was conducted 3 days per week, with all students participating in calisthenics and a 2-mile run as a group. Additional individual

exercise or physical activity was encouraged, and fitness facilities were available for use by the students throughout their training program.

The APFT was conducted on all subjects during the first and eighth week of training. This test consists of three events: push-ups, sit-ups, and a two-mile run. For sit-ups and push-ups, the number of repetitions completed in two minutes was recorded and for the two-mile run, time was recorded. Testing was conducted according to procedures described in FM 21-20, Physical Fitness Training (U.S. Department of the Army, 1992). Raw scores for each event were recorded and later converted to "points" as described in FM 21-20.

ENDURANCE PERFORMANCE AND PEAK OXYGEN UPTAKE

Endurance time to exhaustion (ET)(total time of test in minutes), and peak oxygen uptake (VO₂ peak) were determined during weeks 1 and 8 of OBC using a continuous, uphill, stepwise treadmill protocol originally described by Saltin and Astrand (1967) and modified by Shepard et al. (1968). Following a familiarization period of running on the treadmill and breathing through a mouthpiece, the women began the test at 5 mph, 0% grade for 5 min after which the treadmill grade was increased 2.5% every 3 min to a maximum of 12.5%. If a subject could continue at this stage, speed was increased 0.5 mph every 3 min until exhaustion or volitional fatigue. During the final minute of each 3 min bout of exercise, expired gas collections were made and VO₂ and minute ventilation (VE) were measured using a computerized gas analysis system developed at this Institute. This system consists of a turbine (KL Engineering, Northridge, CA) for measuring expired volume and Applied Electrochemistry S-3A (Ametek, Pittsburgh, PA) and Beckman LB-2 (Sensormedics, Yorba Linda, CA) analyzers for the measurement of expired oxygen and carbon dioxide fractions, respectively. The subject breathed through a mouthpiece and a low resistance, non-rebreathing valve (Hans Rudolph, Inc., Kansas City, KS) into respiratory tubing connected to the turbine. VO2 peak was taken as the highest VO2 recorded during the test, typically that obtained during the final minute of exercise. Heart rate (HR) was continuously monitored electrocardiographically using a modified V5 lead and recorded during the final minute at each level of exercise.

PLASMA VOLUME

Plasma volumes at Week 1 and Week 8 were calculated by using changes in hematocrit and hemoglobin values with the method described by Dill and Costill (1974). Similar calculations have been used in previous studies on exercise-induced change in plasma volume over time (Kanstrup and Ekblom, 1984; Fellman, 1992).

STATISTICAL ANALYSIS

Means and standard deviations of all variables were compared using analysis of variance (ANOVA) to assess pre/post differences in plasma iron levels, mood states (POMS), physiological performance (VO₂ peak and related measures), physical performance (physical fitness test scores), and cognitive performance (four choice reaction time).

Subjects were divided into two groups based on their iron status: normal (Nor) versus depleted iron (Dep). Independent t-tests were used to test differences between the Nor and Dep groups for all dependent variables. This was done separately for both time periods (pre-and post-OBC).

Pre/post change scores for all dependent variables were calculated by subtracting pre-training from post-training values. Mean change scores were compared across post-training iron status with the use of t-tests.

Pearson correlations were computed between the iron indices and all other dependent variables for both pre- and post-OBC time periods. Correlations were also calculated between pre-training iron indices and several demographic variables (e.g., exercise energy expenditure and dietary intake prior to OBC, monthly menstrual iron loss). Multiple regression analyses were used to examine the relationships between serum ferritin concentration and several hematologic and behavioral variables. Unless otherwise indicated, all values shown represent the mean plus or minus standard deviation of measure with p<0.05 as the level of statistical significance.

RESULTS

BIOCHEMICAL MEASURES OF IRON AND NUTRIENT STATUS

Table 3 presents pre- and post-training values for various hematological variables. Initial measures of iron status of women entering active duty revealed that although mean ferritin values were within normal range, 33% of the women had values indicating iron deficiency (<20 μ g/l); 7% had deficient hemoglobin levels (<12 g/dl), indicating anemia. By the completion of OBC, iron deficiency was identified in 64%, almost double the initial finding, and the proportion of women with anemia increased to 13%.

Significant decreases, as indicated in Table 3, were seen in serum iron, ferritin, and transferrin saturation values by the completion of OBC. No significant changes were seen in haptoglobin. Although hemoglobin values did not change significantly, they were at the low end of the normal range.

Table 3. Biological markers of iron and hematologic status (n=45).

Variable	Reference Range	Pre-Training MEAN <u>+</u> SD	Post-Training Mean <u>+</u> SD
Hemoglobin (g/dl)	12.1-17.2	13.4 ± 0.8	13.3 ± 0.8
Hematocrit (%)	36.1-50.3	40.1 ± 2.3	40.0 ± 2.2
Serum iron (µg/dl)	40-150	108.7 ± 50.4	76.6 ± 39.4**
Serum ferritin (µg/l)	20-307	32.7 ± 22.5	21.7 ± 15.4**
Transferrin saturation (%)	15-50	28.3 ± 13.8	21.5 ± 14.0*
Haptoglobin (mg/dl)	16-200	93.8 ± 34.3	99.0 ± 35.1
TIBC (µg/dl)	250-400	393.0 ± 84.5	380.5 ± 66.6

**p<0.01; **p<0.001

NUTRIENT STATUS

Mean pre- and post-training values for metabolism, vitamin and mineral variables are presented in Table 4. Values were within the normal range for all variables at the initial blood draw (except Vitamin C). Vitamin B₁₂ decreased significantly over the eight weeks of training, but still remained within the normal range.

Table 4. Biological markers of metabolism, vitamin and mineral status (n=45).

Variable	Reference Range	Pre-Training Mean <u>+</u> S.D.	Post-Training Mean <u>+</u> S.D.
Vit B ₁₂ (pg/ml)	180-914	413.1 ± 169.6	311.2 ± 119.6*
Serum folate (ng/ml)	2.2-17.3	9.2 ± 4.4	6.8 ± 2.9*
RBC folate (ng/ml)	199-867	396.7 ± 125.4	293.1 ± 98.0*
Magnesium (mg/dl)	1.8-2.5	2.1 ± 0.1	2.0 ± 0.2
RBP ^a (mg/dl)	3.0-6.0	4.2 ± 0.7	4.3 ± 0.7
Vit C (mg/l)	5-20	4.5 ± 2.0	4.5 ± 1.6

^{*}p<0.001

Group means for serum folate values were normal, but declined significantly (p<0.001) over the 8-week training period. A significant decrease was seen in RBC folate levels by week 8, approaching the low end of normal ranges.

Significant negative correlations were found between self-reported exercise levels (kcal/week) prior to active duty and serum ferritin and haptoglobin levels (Table 5). Forty-one of the women indicated they ran at least 3 days per week for an average of 30 minutes per time.

^a Retinol binding protein

Table 5. Correlations between self-reported exercise energy expenditure (kcal/week) prior to active duty and various iron measures (n=54).

Iron Parameter	kcal/week	
Hemoglobin	-0.07	
Ferritin	-0.30*	
Transferrin Saturation	0.11	
TIBCª	0.16	
Haptoglobin	-0.29*	
Iron	0.16	

atotal iron binding capacity

Negative correlations between monthly menstrual cycle variables and ferritin and hemoglobin values are shown in Table 6. Monthly menstrual blood loss, length of menstrual period, and monthly menstrual iron loss showed significant negative correlations with serum ferritin but not with hemoglobin.

Table 6. Correlations between self-reported menstrual cycle variables and pre-training iron measures in female officer basic students (n=56).

Iron Parameter	Length of Menstrual Period (# days)	Monthly Period Blood Loss	Monthly Menstrual
Hemoglobin	-0.13	-0.17	-0.05
Ferritin	-0.28*	-0.30*	-0.33*

^{*}p<0.05

PLASMA VOLUME

Changes in plasma volume were small (6%) over the 8 weeks. No differences were seen between the Nor and Dep groups.

^{*}p<0.05

NUTRIENT INTAKES

Food intake of nutrients prior to entry into active duty (Table 7) was compared with the RDA. Mean reported intakes met or exceeded the RDA for all nutrients except iron and folic acid. Mean energy intake was lower than the RDA. Reported intake during OBC, as assessed by daily diet records, was compared to the MRDA, which reflect increased nutrient needs of military personnel (Table 8). Mean intakes were low for energy (kcal), protein, folic acid, magnesium, and zinc, and the wide range in intakes indicate that intakes of some individuals were suboptimal. Only 25% of the subjects consumed coffee and 14% consumed tea on a daily basis (inhibitors of iron absorption). Orange juice (enhancer of iron absorption) was consumed on a daily basis by 12% of the subjects.

Table 7. Mean reported pre-training nutrient intakes of female soldiers attending officer basic training (n=50).

Nutrient	RDA ¹	FFQ Intake ²	%RDA
Energy, kcal	2200	1608 ± 659 ³	73
Protein, g	50	73 ± 29	146
Fat, g	4	53 ± 32	
Saturated fat, g		18 ± 12	-
Carbohydrate, g		211 ± 83	-
Vitamin C, mg	60	163 ± 106	272
Thiamin, mg	1.1	1.6 ± 0.8	145
Riboflavin, mg	1.3	2.2 ± 1.0	169
Niacin, mg	15	20 ± 10	133
Vitamin B ₆ , mg	1.6	1.9 ± 0.9	119
Folic Acid, mcg⁵	400	361 ± 209	90
Vitamin B ₁₂ , mcg	2.0	6	-
Vitamin A, RE	800	1193 ± 507	149
Calcium, mg	800-1200	972 ± 544	81
Phosphorus, mg	800-1200	1297 ± 501	108
Magnesium, mg	280	577 ± 463	206
Iron, mg	15	12.6 ± 5.6	84
Zinc, mg	12	12.7 ± 7.4	106
Potassium, mg	7	2693 ± 1018	
Sodium, mg	8	2884 ± 985	
Dietary Fiber, g	_9	11.6 ± 5.4	
Cholesterol, mg	10	197 ± 109	

¹Recommended Dietary Allowances; ²Food Frequency Questionnaire; ³ mean <u>+</u> SD; ⁴No RDA established denoted by "--"; ⁵As recommended by the CDC, 1992; ⁶Data not recorded denoted by "-"; ⁷Estimated safe and adequate intake is 1875-5625 mg of potassium; ⁸Target for sodium is 1700 mg per 1000 kcal (i.e., 4080 for females); ⁹Recommended dietary fiber intake is 20-30 g; ¹⁰Suggested cholesterol maximum intake is 300 mg.

Table 8. Mean dietary intakes of female soldiers during officer basic training (n=50)1.

Nutrient	MRDA ²	Dietary Intake	%MRDA
Energy, kcal	2400	2037 ± 56 ³	85
Protein, g	80	75 ± 15	94
Fat, g	_4	63 ± 17	
Saturated fat, g		23 ± 7	
Carbohydrate, g		289 ± 70	
Vitamin C, mg	60	115 ± 64	192
Thiamin, mg	1.2	1.7 ± 0.6	142
Riboflavin, mg	1.4	2.1 ± 0.7	150
Niacin, mg	16	24 ± 8	150
Vitamin B ₆ , mg	2.0	2.0 ± 0.8	100
Folic Acid, mcg⁴	400	342 ± 156	86
Vitamin B ₁₂ , mcg	3.0	4.6 ± 2.3	153
Vitamin A, RE	800	1259 ± 961	157
Calcium, mg	800-1200	918 ± 317	115
Phosphorus, mg	800-1200	1333 ± 323	167
Magnesium, mg	300	285 ± 80	95
Iron, mg	15	16.8 ± 6.9	112
Zinc, mg	15	11.2 ± 0.6	75
Potassium, mg	_6	2666 ± 738	
Sodium, mg	_7	3439 ± 841	
Dietary Fiber, g	_8	15.4 ± 5.5	
Cholesterol, mg	_9	201 ± 90	

¹Does not include vitamin, mineral, or other supplements; ²Military Recommended Dietary Allowances; ³mean ± SD; ³No MRDA established denoted by "--"; ⁴As recommended by the CDC, 1992; ⁶Estimated safe and adequate intake is 1875-5625 mg of potassium; ⁷Target for sodium is 1700 mg per 1000 kcal (i.e., 4080 for female soldiers); ⁸Recommended dietary fiber intake is 20-30 g; ⁹Suggested cholesterol maximum intake is 300 mg.

Individual intake is more clearly depicted in Table 9, which presents the number of women with mean nutrient intakes at selected levels of the MRDA (<60%, 60-69%, 70-79%, 80-89%, 90-99% and ≥ 100%). Supplement intake is not included in calculation of dietary intake of nutrients. Fifty-one percent of the women reported taking multivitamin supplements; in addition, 21% took Vit C , 9% took Vit E , 5% took Vit B complex, 12% took calcium, and 12% took iron.

Table 9. Number of female soldiers with mean nutrient intakes at selected levels of the MRDA (n=50).

Percent of MRDA									
Nutrient	<60	60-69	70-79	80-89	90-99	<u>≥</u> 100			
Kilocalories	2	6	12	17	2	11			
Protein	2	1	11	7	9	20			
Vitamin C	2	4	2	0	0	42			
Thiamin	0	1	1	4	1	43			
Riboflavin	0	1	0	3	2	44			
Niacin	0	0	1	2	1	46			
Vitamin B ₆	6	4	5	5	2	28			
Folic Acid	13	4	4	4	3	22			
Vitamin B ₁₂	2	1	3	5	1	38			
Vitamin A	6	0	3	3	4	34			
Calcium	1	2	8	6	1	32			
Phosphorus	0	0	0	1	0	49			
Magnesium	4	4	5	8	8	21			
Iron	2	0	6	11	6	25			
Zinc	14	7	6	4	4	15			

Food group sources of dietary iron are shown in Figure 1. The major contributor of dietary iron in this population was from the bread and grain category, with the meat category providing the second highest proportion. Breakdowns of the two main contributors of dietary iron - meats and grains - are shown in Figure 2 and Figure 3. Breakfast cereals provided the largest proportion of iron from the grain group; the mixed dishes were the major contributor of iron in the meat group.

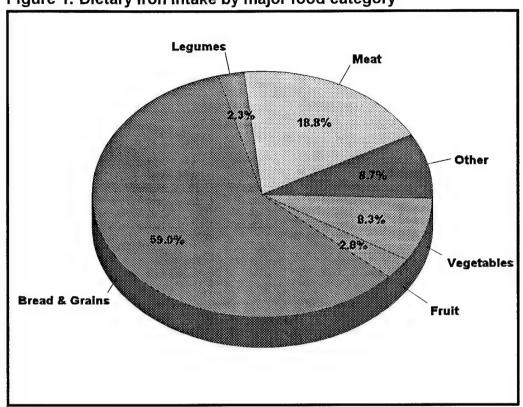
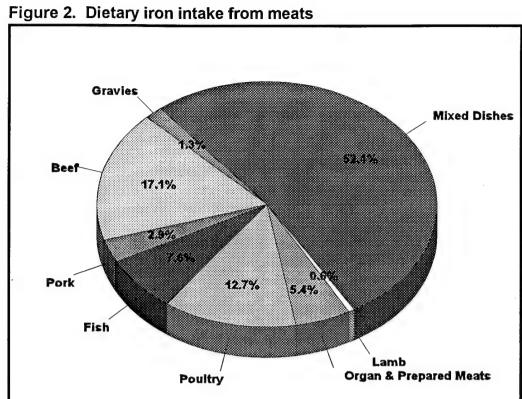


Figure 1. Dietary iron intake by major food category



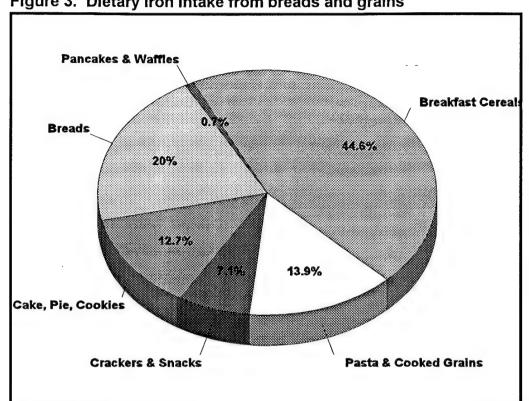


Figure 3. Dietary iron intake from breads and grains

EFFECT OF BASIC TRAINING ON BODY COMPOSITION, PHYSIOLOGICAL STATUS, AND PHYSICAL PERFORMANCE

Table 10 presents the mean pre- and post-training values for the body composition, maximal treadmill test, and APFT variables.

Table 10. Mean values for body composition, maximal treadmill test, and the APFT, pre-and post-training (mean \pm SD).

Variable	Pre-training	Post-training
Body Composition, n=45		
Body mass, kg	59.6 ± 8.1	60.1 ± 7.5*
Body fat, %	25.6 ± 3.4	25.8 ± 3.4
Fat free mass, kg	44.0 ± 4.9	44.4 ± 4.7**
Treadmill performance, n=45		
Peak VO ₂ , I•min ⁻¹	2.78 ± 0.45	2.93 ± 0.42***
Peak VO ₂ , ml•kg ⁻¹ •min ⁻¹	47.0 ± 6.6	48.9 ± 5.8***
VE, I•min ⁻¹	92.7 ± 18.7	101.8 ± 13.1**
HR, b•min ⁻¹	193 ± 9	193 ± 9
Endurance Time, min	15.75 ± 4.40	17.50 ± 4.00***
APFT, n=51		
Pushups, #/2 min	39.3 ± 17.9	45.9 ± 15.8***
Situps, #/2 min	66.5 ± 21.3 77.4 ± 16.6*	
Two-mile run, min	17.90 ± 2.63 17.05 ± 1.83***	
Total Score, points	242 ± 49	268 ± 31***

^{*}p<0.05; **p<0.01; ***p<0.001

There was a significant increase in body mass (p<0.05) and fat-free mass (p<0.01). Measures of physical performance from the maximal treadmill test and the APFT increased significantly from pre-to post-training. VO₂ peak expressed in absolute terms (I•min⁻¹) and relative to body mass (mI•kg⁻¹•min⁻¹) increased between 4% and 5%. ET however, increased nearly 11% representing a significant improvement in aerobic

performance. This demonstrates that while these two variables are highly correlated (r=0.84, p<0.01), a running program can improve endurance performance despite producing a relatively small change in aerobic capacity.

EFFECT OF IRON DEPLETION ON PHYSICAL PERFORMANCE Pre-Training Data

Table 11 compares mean body composition, treadmill performance, and APFT data between subjects grouped by serum ferritin levels: those with normal ferritin (≥20.0 µg/l) and those with iron depletion (ferritin <20.0 µg/l). There were no significant effects of iron depletion at the beginning of training on any of the measured variables.

Table 11. Body composition, treadmill performance, and the APFT variables in normal (Nor) and iron depleted (Dep) subjects (mean ± SD), pre-training.

Variable	Nor	Dep
Body Composition	n = 32	n = 18
Body mass, kg	61.3 ± 7.5	57.3 ± 8.3
Body fat, %	27.0 ± 3.5	24.3 ± 2.5
Fat free mass, kg	44.8 ± 4.5	43.3 ± 5.4
Treadmill Performance	n = 32	n = 18
Peak VO ₂ , I•min ⁻¹	2.80 ± 0.38	2.72 ± 0.54
Peak VO ₂ , ml•kg ⁻¹ •min ⁻¹	46.0 ± 6.8	47.5 ± 7.0
VE, I•min ⁻¹	90.1 ± 20.1	95.2 ± 11.2
HR, b•min ⁻¹	194 ± 9	192 ± 8
Endurance Time, min	15.14 ± 4.55	15.89 ± 4.50
APFT	n = 38	n = 18
Pushups, #/2 min	36.4 ± 15.1	44.4 ± 20.6
Situps, #/2 min	63.6 ± 21.0	73.6 ± 21.9
Two-mile run, min	18.11 ± 2.45	17.53 ± 2.79
Total Score, points	240 ± 47	249 ± 50

Correlation coefficients between the various serum constituents and the performance variables from the maximal treadmill test and the APFT are shown in Table 12. No significant relationships were seen between any of the hematological and maximal exercise performance variables.

Table 12. Correlation coefficients between maximal performance indicators and various serum constituents, pre-training.

Markers	VO₂peak, I•min	End Time, min	Situps, #/2 min	Pushups, #/2 min	Two-mile run, min
Hemoglobin	-0.06	-0.09	-0.18	-0.13	0.20
Ferritin	-0.17	-0.15	-0.33	-0.23	0.13
Transferrin	0.06	0.02	0.06	0.00	0.01
TIBC	-0.07	-0.04	0.10	0.13	0.01
Iron	0.02	-0.01	0.08	0.04	0.03

Post-Training Data

The mean values for the body composition, maximal treadmill test, and APFT variables for subjects with normal iron status and those with iron depletion at the end of training are presented in Table 13.

Table 13. Body composition, treadmill performance, and the APFT variables in normal (Nor) and iron depleted (Dep) subjects (mean \pm SD), post-training.

Variable	Nor	Dep
Body Composition	n = 17	n = 26
Body mass, kg	61.4 ±7.2	59.2 ±7.1
Body fat, %	27.3 ±4.1	25.1 ±2.7
Fat free mass, kg	44.3 ±3.9	44.4 ±5.3
Treadmill Performance	n = 17	n = 26
Peak VO ₂ , I•min ⁻¹	2.93 ±0.39	2.92 ±0.45
Peak VO ₂ , ml•kg ⁻¹ •min ⁻¹	48.1 ±6.4	49.4 ±5.6
VE, I•min ⁻¹	101.3 ±13.2	102.8 ±13.6
HR, b•min ⁻¹	192 ±8	194 ±9
Endurance Time, min	17.16 ±4.71	17.74 ±3.70
APFT	n = 18	n = 27
Pushups, #/2 min	43.3 ±18.3	48.3 ±14.9
Situps, #/2 min	71.8 ±19.3	80.0 ±15.2
Two-mile run, min	17.00 ±2.07	16.90 ±1.76
Total Score, points	265 ±33	271 ±31

Again, as seen in the pre-training period, there were no differences in any of these variables between women who were iron-depleted and those who had normal levels of ferritin.

In addition, there were no significant correlations between the various blood constituents and indicators of maximal exercise performance as shown in Table 14.

Table 14. Correlation coefficients between maximal performance indicators and various serum constituents, post-training.

Markers	VO₂ peak, I•min	End Time, min	Situps, #/2 min	Pushups, #/2 min	Two-mile run, min
Hemoglobin	-0.03	0.03	-0.24	-0.18	0.06
Ferritin	-0.14	-0.10	-0.23	-0.14	0.01
Transferrin	-0.09	-0.01	0.02	-0.03	-0.01
TIBC	0.18	0.13	0.18	0.24	-0.21
Iron	0.05	0.13	0.14	0.14	-0.16

Pre-to-post training differences

Table 15 presents the mean data for body composition, maximal treadmill, and APFT variables for subjects divided into three groups: those who had normal serum ferritin levels in both the pre- and post-training period (Nor/Nor); those with normal levels pre-training but who became iron depleted over the eight weeks of training (Nor/Dep); and those who were iron depleted both pre-and post-training (Dep/Dep). There were no changes in any of the variables in Table 15 over the eight weeks for any of the three groups.

Table 15. Mean values for body composition, treadmill performance and APFT variables for subjects categorized by iron status in the pre/post-training periods (mean ±SD).

Variable	Nor/Nor	Nor/Dep	Dep/Dep
Body Comp	n = 16	n = 9	n = 17
Body mass, kg	61.7 ±7.7	61.0 ±7.0	58.3 ±8.2
Body fat, %	27.5 ±4.2 ^a	26.2 ±2.9 ^{a,b}	24.4 ±2.4 ^b
Fat free mass, kg	44.4 ±4.0	45.4 ±5.1	43.9 ±5.5
Treadmill Performance	n = 16	n =9	n =17
Peak VO ₂ , I•min ⁻¹	2.93 ±0.40	3.04 ±0.33	2.85 ±0.50
Peak VO ₂ , ml•kg ⁻ ¹ •min ⁻¹	47.7 ±6.4	50.3 ±6.1	49.0 ±5.4
VE, I•min⁻¹	101.3 ±13.6	107.2 ±13.9	100.5 ±13.3
HR, b•min ⁻¹	191 ±9	197 ±5	192 ±9
End Time, min	16.82 ±4.64	18.83 ±3.38	17.16 ±3.82
APFT	n = 17	n = 11	n = 16
Pushups, #/2 min	42.1 ±18.0	49.1 ±12.2	47.8 ±16.9
Situps, #/2 min	70.8 ±19.4	79.3 ±15.7	80.6 ±15.3
Two-mile run, min	17.10 ±2.08	16.99 ±1.50	16.84 ±1.97
Total Score, points	263 ±34	279 ±28	266 ±33

Different letters denote statistical significance, p<0.05

MOOD STATES

Effect of Officer Basic Training

Mood profiles of these subjects exhibited the flattened iceberg profile (Figure 4). The iceberg profile is characterized by the five negative mood scales having T-scores lower than college normative values and vigor, the one positive mood scale, having a T-score higher than the college norm (Morgan, 1985). Post-training levels of subjective fatigue were significantly higher (p<0.05) than pre-training. Total mood scores were also significantly poorer (p<0.05) following training. Although other individual mood factors did not show significant changes, consistent trends were observed with negative moods (tension, depression, anger, and confusion) increasing and the lone positive factor (vigor) decreasing. These changes in the individual mood factors contributed to the significant change in total mood scores. Table 16 presents the means and standard deviations pre- and post-training with level of significance. Weak but significant correlations existed (p<0.05) between level of self-reported exercise and vigor (R=0.28), self-reported exercise and fatigue (R=-0.29), and self-reported exercise and total mood (R=0.29) pre-training.

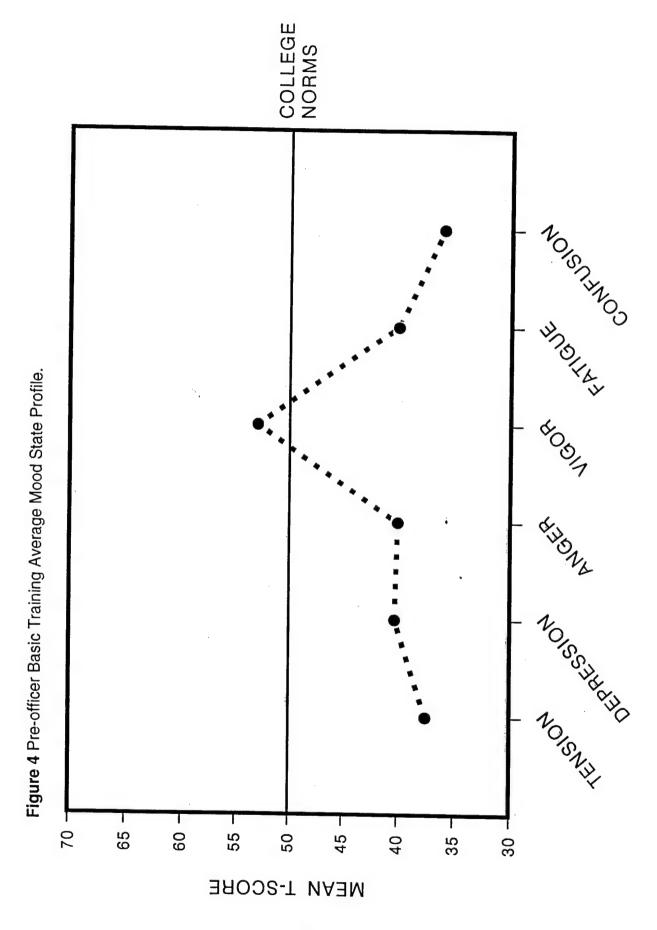


Table 16. Profile of Mood States (POMS) raw score measures pre- and post-training in 45 female officers.

	Pre-Training MEAN ± S.D.	Post-Training MEAN ± S.D.	t	р
Tension	4.60 ± 3.35	5.18 ± 4.09	0.90	NS
Depression	3.36 ± 3.64	4.62 ± 5.72	1.49	NS
Anger	2.09 ± 3.14	3.20 ± 4.77	1.44	NS
Vigor	17.22 ± 6.02	15.33 ± 7.88	1.69	NS
Fatigue	4.09 ± 4.06	7.18 ± 6.36	3.51	0.01
Confusion	3.84 ± 2.70	4.13 ± 2.92	0.61	NS
Total Mood	99.24 ± 16.33	91.02 ± 25.15	2.25	0.05

Effect of Iron Status

Pearson product-moment correlations post-training showed a weak but significant positive correlation (R = 0.33) with iron status and POMS confusion. After classifying individuals as either Nor or Dep as described previously, no significant differences were seen pre-training or post-training. Furthermore, no differences in mood state change scores by post-training iron status were observed. There were no differences in mood post-training in those classified as Dep versus Nor pre-training. There were also no differences between those who had normal status (Nor/Nor) throughout versus those who moved from normal to depleted (Nor/Dep) versus those who remained depleted (Dep/Dep). Means and standard deviations by these groups are shown in Table 17.

Table 17. Profile of Mood States (POMS) scales by iron status pre/ post- training in 42 female officers.

	Nor/Nor (n=16)	Nor/Dep (n=9)	Dep/Dep (n= 17)
Tension	5.63 ± 5.39	5.89 ± 3.69	4.82 ± 3.15
Depression	5.50 ± 6.08	5.00 ± 4.42	4.18 ± 6.50
Anger	3.50 ± 4.55	4.33 ± 5.89	2.53 ± 4.85
Vigor	16.43 ± 9.57	14.22 ± 8.41	14.76 ± 6.85
Fatigue	6.69 ± 6.92	8.11 ± 6.95	7.24 ± 6.28
Confusion	3.81 ± 3.35	5.44 ± 3.43	4.06 ± 2.30
Total Mood	91.31 ± 31.03	85.44 ± 25.45	91.94 ± 21.70

Weight Loss Status

Significant differences in pre-training tension (p<0.01) were seen for those trying to lose weight versus those that were not. Those trying to lose weight were more distressed on all mood factors compared to those that were not trying to lose weight. Table 18 shows the means and standard deviations by weight loss group.

Table 18. Pre-training Profile of Mood States (POMS) raw score measures by weight loss efforts in 45 normal-weight female officers.

	Trying to Lose Wt. MEAN ± S.D. (n = 17)	Not Trying to Lose Wt. MEAN ± S.D. (n = 28)	t	р
Tension	6.53 ± 3.28	4.00 ± 3.16	2.54	<.05
Depression	5.06 ± 5.06	2.43 ± 2.15	2.04	NS
Anger	2.88 ± 3.94	1.29 ± 1.51	1.60	NS
Vigor	17.35 ± 7.09	16.25 ± 5.32	0.55	NS
Fatigue	4.35 ± 5.02	4.39 ± 3.45	0.03	NS
Confusion	4.94 ± 3.65	3.50 ± 1.86	1.51	NS
Total Mood	93.59 ± 22.49	100.64 ± 11.67	1.20	NS

FOUR-CHOICE REACTION TIME

Effect of Officer Basic Training

Post-training latency of correct hits was significantly shorter (p<0.0001) than pretraining scores. Time out errors were also significantly lower (p<0.05) following training. No other reaction time measures showed significant changes pre- to posttraining (Table 19).

Table 19. Four-choice reaction time measures pre- and post-training in 45 female officers.

	Pre-Training MEAN ± S.D.	Post-Training MEAN ± S.D.	t	р
Correct Hits Total = 250	237.51 ± 27.06	241.64 ± 6.59	1.04	NS
Correct Hits Latency (ms)	515.20 ± 64.64	471.20 ± 57.31	8.73	0.0001
Incorrect Hits (Total)	8.64 ± 9.36	8.40 ± 6.65	0.22	NS
Incorrect Hits Latency (ms)	231.03 ± 103.10	200.16 ± 86.64	1.84	NS
Premature Errors (Total)	0.42 ± 0.87	0.44 ± 0.73	0.18	NS
Time-out Errors (Total)	0.24 ± 0.57	0.09 ± 0.36	2.20	0.05

Effect of Iron Status

No correlation existed between iron measures and any of the four-choice reaction time measures or in change scores of iron status and change scores of the various reaction time measures. There were no significant differences pre-, or post-training between iron classification groups. In addition, no differences were seen between iron groups in change scores from pre- to post-training for any of the four-choice reaction time measures. Finally, in examining post-training four-choice reaction time performance, no differences were seen in those classified as Dep versus Nor prior to the start of training, as well as no differences between Nor/Nor, Nor/Dep, or Dep/Dep. Means and standard deviations by these groups are shown in Table 20.

Table 20. Post-basic four-choice reaction time by iron status pre/post-training in 42 female officers.

	Nor/Nor (n=16)	Nor/Dep	Dep/Dep (n= 17)
Correct Hits	241.6 ± 8.2	240.7 ± 8.0	242.1 ± 4.4
Latency Correct Hits (msec)	479.3 ± 67.5	474.1 ± 37.8	456.1 ± 46.7
Incorrect Hits	8.6 ± 8.3	9.3 ± 8.0	7.9 ± 4.4
Latency Incorrect Hits (msec)	186.9 ± 72.3	206.6 ± 118.3	215.3 ± 80.3
Premature Errors	0.7 ± 1.0	0.1 ± 0.3	0.5 ± 0.6
Time-Out Errors	0.1 ± 0.3	0.0 ± 0.0	0.2 ± 0.5

DISCUSSION

BIOCHEMICAL MEASURES OF IRON AND NUTRIENT STATUS

Although mean values of iron status for these women were within normal ranges prior to training, and neither hemoglobin nor hematocrit demonstrated declines over the course of training, several markers did show significant decreases. The dramatic drop in serum ferritin was an indication that iron stores were being depleted as training progressed. The doubling over the eight weeks of officer basic training of the proportion of women with iron depletion from 33% to 64% and those with anemia from 7% to 13% is a cause for concern. When mean values were compared with those of women entering enlisted basic training in 1993 (Westphal et al., 1995), similar decreases were seen in iron markers for both populations (Table 21). Iron status was similar for enlisted women entering active duty at the same time as the officers in this study (Cline and Pusateri, 1996); post-training values for enlisted women are not available for comparison. A period of eight weeks of training resulted in no significant changes in haptoglobin, indicating that red cell hemolysis was most likely not a problem with this population.

Table 21. Comparisons of biological markers of iron status - women entering active duty for training.

	Officers 1	995(n=45)	Enlisted 1993(n=158)		Enlisted 1995(n=43)
Marker	Pre-training	Post-training	Pre-training	Post-training	Pre-training
Hemoglobin	13.4 ± 0.8	13.3 ± 0.8	12.9 ± 1.0	12.8 ± 1.1	13.2 ± 1.0
Ferritin	32.7 ± 22.5	21.7 ± 15.4***	17.4 ± 14.2	10.9 ± 9.1*	33.9 ± 28.1
Transferrin Sat (%)	28.3 ± 13.8	21.5 ± 14.0**	22.9 ± 10.6	22.8 ± 11.9	21.8 ± 10.4
TIBC	393.0 ± 84.5	380.5 ± 66.6	341.5 ± 47.6	335.2 ± 46.5	370.8 ± 47.8

^{*}p<0.05, **p<0.01, ***p<0.001, differences between pre- and post-training status

The negative correlations found between exercise and serum ferritin, as well as haptoglobin (Table 5), are similar to those reported in other studies on women participating in sports activities and physical exercise (Clement and Asmundson, 1982; Blum et al., 1986; Haymes and Spillman, 1989; Rajaram et al., 1994; Pratt et al., 1996). The small change in plasma volume indicated that measurements of iron status were not likely affected by plasma volume expansion associated with exercise or acclimatization to heat.

Depressed immune function has been seen in iron deficiency, even before the development of anemia. This appears to be related to the level of tissue iron stores, and may precede development of frank anemia in individuals who are iron deficient (Dhur et al., 1989). Immune function was not assessed in this study, but should be an area of future investigation, as there is concern of increased illness rates in students in training environments.

Decrements in physical or mental performance from iron depletion were not seen in this study, but this may be because of the short duration of the study. These results are similar to previously reported studies (Celsing et al., 1986; Risser et al., 1988) in which exercise and iron status were not correlated with performance. What has not been measured is how long iron stores remain depleted after training and the impact on future work performance.

Folate and iron deficiencies often occur simultaneously. The significant decline seen in folate status for these subjects over the 8-week training period may reflect a significant change in eating patterns. Negative folate balance is manifested by depression of serum folate concentrations, which may occur after only 3 weeks of dietary folate deprivation (O'Connor, 1991). Therefore, serum folate concentrations typically reflect recent folate intake. The presence of depressed red blood cell (RBC) folate concentrations are likely indicative of biochemical folate deficiency. Depression of these concentrations occurs around 17-19 weeks of negative folate balance, and indicates a severe depletion of folate stores. Although folate concentrations did not reach severe depletion levels, the dramatic decrease in folate status, however, is of concern because this population is of childbearing age.

Evaluation of other nutritional markers indicates that the nutritional status of this population was good upon entry into training.

NUTRITIONAL INTAKES

Reported nutrient intakes prior to active duty are similar to those from a survey of women entering enlisted basic training at the same time as this population of officers (Cline and Pusateri, 1996). Energy intake was lower than allowances, which may reflect a desire to meet weight standards.

One factor that most likely played an important role in decreased iron status was that our population did not have access to adequate cooking facilities, as evidenced by the foods recorded on diet logs. These women ate an abundance of convenience foods, restaurant foods, and snacks. In this aspect, they differed from enlisted women in basic training who were required to consume all their meals in military dining facilities that do not offer short order or snack items.

Of concern is that, as eating habits changed upon entry to active duty (Table 8), a majority of these women did not meet dietary allowances for energy, protein, magnesium, iron, and zinc. Because of the physical demands imposed by military training and the importance of nutrition on physical performance, the deficit of these nutrients in military women is of particular concern. The desire to lose weight to meet body weight standards, as mandated by the U.S. Army Weight Control Program (U.S. Department of the Army, 1994), may have been one reason for low energy intakes reported by 78% of these women. With inadequate energy intake, it is difficult to consume enough foods to meet the nutritional requirements of other nutrients, including iron.

Dietary iron intake was low in 50% of women. As indicated earlier, a major food contributor was the grain and cereal group, containing non-heme iron with limited absorption. Non-heme absorption is inhibited by tannic acid (in tea), coffee, phytic acid (in wheat bran), phosphates, antacids, and calcium salts (Haymes, 1993; Finch and Huebers, 1982; Morck et al., 1983; Fairbanks, 1994). While dietary vitamin C intake was above 100% of the MRDA in a majority of these women, the enhancement

potential of vitamin C may have been limited by beverage selection and intake of whole grains. Meats contributed only 18.8% of dietary iron compared to 31% reported in a national nutrition survey (Murphey and Calloway, 1986). Because most iron came from non-heme sources in our population, intestinal absorption would not have been as high as that from diets with higher intakes of heme iron.

When compared to an iron intake of 12.4 ± 0.4 g reported from food consumption data in NHANES III (Interagency Board, 1995), mean iron intake (16.2 ± 3.5 g) for the current study group was higher than that of women in the general population, but was similar to that of women in enlisted basic training (16.8 ± 6.9 g) (Westphal, 1995). Studies on female athletes have reported intakes of dietary iron ranging from 10.1 mg/day in field hockey players (Diehl et al., 1986) to 20.6 mg/day in cross-country skiers (Haymes et al., 1986). Surveys of the nutrient intake of female athletes suggest that the average female athlete has a slightly higher iron intake, 12-18 mg/day, than women in the general population (Haymes and Spillman, 1989).

In this study, serum markers of iron status were not significantly related to short-term dietary iron intakes, results similar to those reported in previous studies on women in enlisted basic training (Klicka et al., 1993; Westphal et al., 1995). The amount of iron absorbed, however, may have been inadequate because of the low percentage of heme iron food sources consumed by this population.

PHYSICAL ACTIVITY PATTERNS

Physical activity patterns reported by this group reflect an energy expenditure level that is quite high when compared with the general population of comparable age (Dishman and Steinhardt, 1988). They are similar to reported increases of regular endurance exercise activities for women (Pate et al., 1990), but a much greater percentage of our population participated at levels thought to promote or maintain cardiorespiratory fitness than those previously reported (Caspersen and Merritt,1995). Intense exercise has been shown to decrease iron stores because of sweat and stool losses (Smith et al., 1985). The high summer temperatures and humidity in San Antonio during training may have contributed to sweat losses of iron in these women.

BODY COMPOSITION, PHYSIOLOGICAL STATUS, AND PHYSICAL PERFORMANCE

The major findings of this study concerning physical performance were: 1) the physical fitness of the subjects entering basic training was well above average for comparably aged servicewomen and improved significantly during the eight weeks of training, and 2) there was no effect of iron depletion either assessed cross-sectionally during the pre-training period or as a consequence of the eight weeks of basic training on any measure of physical performance.

The increase in body mass after the eight weeks of training, while statistically significant, was very small and most likely reflects the similarly small increase in fat-free mass since there was no change in percent body fat. The values for body mass, fat-free mass and percent body fat in the pre-training period are very similar to those reported for a large age-matched Army population (Fitzgerald et al., 1986).

Aerobic power represents one of the basic components of physical fitness and plays a decisive role in the performance of such endurance activities as running and prolonged load carriage. The most common and objective measure of aerobic power is maximum or peak oxygen uptake which provides an indirect assessment of the capacity for energy generation from aerobic sources. The higher the peak oxygen uptake, the higher is the aerobic energy output and the greater the aerobic performance of the individual. In this study, the mean peak oxygen uptake of the women at the beginning of training exceeded by nearly 20% levels reported for enlisted women entering basic training (Patton, et al., 1980; Vogel et al., 1986) and by 6-8% levels reported more recently from various samples of active duty servicewomen (Patton, et al., 1995; Nindl, et al., 1998). Indeed, the aerobic fitness of the present group was nearly identical to that reported by Daniels et al. (1979) for women entering the U.S. Military Academy at West Point (VO₂max = 46.0 ml•kg⁻¹•min⁻¹). In a survey of physical fitness of NATO forces, it was reported that the aerobic power of various female military populations entering initial recruit training ranged between 38-41 ml·kg⁻¹·min⁻¹ (NATO Defense Research Group, 1986). Thus, the aerobic fitness of these women entering the Army was higher than that of any previously reported group of military women.

In addition, the performance of these women on the three-event APFT at the beginning of training was equally impressive. The mean values achieved for each event, as well as the total score for the three events combined, were similar to those reported for comparably aged women who had been on active duty for several months and, therefore, had been participating in regular unit and individual physical training (Knapik et al., 1994). Another indication of the relative physical fitness of this group is seen in a comparison of the individual events and the total APFT score to those of women entering enlisted basic training at Ft. Jackson, SC (Westphal et al., 1995). In that group of 174 women, the mean number of situps and pushups was 31 and 8, respectively, versus 67 and 39 for officers, and the two-mile run time was approximately 20 minutes for enlisted versus 18 minutes for officers. Additionally, the mean total score on the APFT was less than half that seen in the present study.

It is well established that aerobic exercise training can increase maximal or peak oxygen uptake. The 4% increase in VO₂ peak in the present study (expressed in ml•kg⁻¹•min⁻¹), while statistically significant, represents a relatively modest change in aerobic fitness suggesting that the training stimulus (i.e., the intensity, duration, and frequency of the training) was less than adequate to promote a significant training response. However, because of the high initial aerobic power of these women, this level of increase was not totally unexpected. In similar studies, Patton et al. (1980) found women to increase approximately 7% (from 37.0 to 39.3 ml•kg⁻¹•min⁻¹) during eight weeks of enlisted basic training, while Daniels et al. (1979) demonstrated an 8% increase in VO₂ peak (46.0 to 49.7 ml•kg⁻¹•min⁻¹) of female cadets after seven weeks of training. This study demonstrates that while VO₂ peak and ET are highly correlated (r=0.84, p<0.01), a running program can improve endurance performance despite producing a relatively small change in aerobic capacity.

The results of the training were also reflected by the significant increase in performance on the two-mile run: the mean time decreased approximately 5% over the eight weeks. These data agree closely with the nearly 60 sec improvement in two-mile run times reported in the study by Daniels et al. (1979). The change in the two-mile run performance in the present study also agrees with the change seen in VO₂ peak: the correlation between the two was 0.83. Indeed, the relationship between $\dot{V}O_2$ max and

run times for various distances has been shown to exceed 0.80 (Knapik, 1989).

The training program, despite the relatively small improvement in VO₂ peak, did result in an 11% increase in run time to exhaustion during the maximal treadmill test. This increase is highly significant and is likely due to a combination of the small increase in VO₂ peak as well as an improved running economy and a decrease in the percent of aerobic capacity utilized during the test. While these latter two variables were not measured, it is recognized that these three variables represent the primary determinants of running performance (Sparling, 1984). Thus if a training program results in little or no improvement in VO₂ peak, running performance may still improve significantly due to an improved efficiency of running (running economy) and/or to a lower percentage of VO₂ peak used during the performance.

The effects of severe iron deficiency and anemia on the metabolic and physiological responses during exercise have been studied extensively (Viteri and Torun, 1974; Gardner et al., 1977; Clement and Sawchuk, 1984). More recently, however, there has been an interest in determining the influence of iron deficiency without overt anemia on energy metabolism and exercise performance. Mild and moderate iron deficiency (characterized by reductions in iron storage pools in tissues, in circulating iron, and in transport iron concentrations) have been shown to affect work metabolism. Nilson et al. (1981) suggested that iron deficiency without anemia in athletes can reduce physical work capacity and lead to excess lactate production as demonstrated by significantly lower maximum lactate levels for a given workload following some replacement of body iron stores with 10-14 days of iron therapy. Similarly, Schoene et al. (1983), in his study of iron-deficient women without overt anemia and supplemented with iron, showed no change in peak oxygen uptake, but a reduction in the concentration of circulating lactate. More recently, Lukaski et al. (1991) reported that iron depletion (mean ferritin levels of 6 µg/l) in women of similar age to those in the present study was associated with a reduced rate of oxygen utilization, total oxygen uptake, and elevated post-exercise concentration of lactate. However, these authors reported no effect on peak oxygen uptake or exercise duration. It was thus concluded that while iron depletion has no influence on the oxygen carrying capacity, there are effects on muscle metabolism during exercise.

The data clearly show that the level of iron depletion that occurred in the subjects in this study had no significant effect on any measure of physical performance. The only significant difference among the three groups was in the percentage of body fat which was lower in individuals who were iron depleted prior to training and who remained iron depleted following training compared to the other two groups.

The findings by Lukaski et al. (1991) on VO₂ peak and exercise endurance support those of the present study in which iron depletion, as reflected by serum ferritin levels below 20 μg/l, resulted in no effect either cross-sectionally or longitudinally on peak exercise performance, endurance time to exhaustion or aerobic and anaerobic performance as measured by the APFT. A recent study by Westphal et al. (1995) who determined the relationship between iron status and physical performance in female soldiers during eight weeks of enlisted basic training demonstrated that iron depletion did not affect either aerobic or anaerobic performance as measured by the APFT.

The available data suggest that despite serum ferritin levels below 20 µg/l being associated with the near complete depletion of bone marrow iron (Clement and Sawchuk, 1984), there is no significant impact on the maximal transport of oxygen as measured by peak oxygen uptake, on endurance performance as measured by treadmill time to exhaustion or two-mile run time, and on anaerobic or muscular endurance type activities as measured by timed situps and pushups.

MOOD STATES

Measures of mood were taken in a rested state during Weeks 1 and 8 of training at approximately the same time during the day. Mood profiles were similar to the iceberg profile that characterizes many athletic populations with vigor (the lone positive mood state) above college norms and the five negative scales below college norms (Morgan, 1985; Morgan et al., 1987b). The difference in our population was that vigor was somewhat reduced with a T-Score of 53 as opposed to the more typical athletic population T-Score of 60. The moods in this study however, matched almost exactly the flattened iceberg profile reported by Johnson and Merullo (1997) in male U.S. Army soldiers.

There was a significant increase in subjective fatigue at Week 8 of training. Other mood scales all followed the same pattern of increased distress even though these changes did not reach significance at the set testing level. It is possible that the activities of the particular day of testing were more fatiguing. However, more than likely, the fatigue reported was based on the continuous physical and psychological stress the training program imposed. Previous research with Ranger training saw increases in mood disturbances (Bugge et al., 1979). Mood change as a result of basic training has not been examined in either men or women. There was no relationship between iron status and mood states, which is similar to previous reports (Risser et al., 1988; Edgerton et al., 1979).

FOUR-CHOICE REACTION TIME

Reaction-time measures were not affected by iron status. The limited previous research on iron deficiency and cognitive function primarily examined deficiencies in children undergoing maturation where deficits in iron were greater than in the present study (Beard, 1995; Pollitt, 1993; Walter, 1993). The need for sufficient iron sources for proper cognitive functioning and development has been established. However, the effects of short-term iron depletion due to physical stress, a change in diet, or blood loss has not been documented. It is also not clear what effect iron deficiency has on cognitive functioning in those who are past the brain growth phase of life. Based on these results, it is unlikely that iron supplementation would improve cognitive performance in the short term. The effect of long-term iron depletion still warrants further investigation.

CONCLUSIONS

Women entering active duty for officer basic training were more physically fit than enlisted women of the same age who have been previously assessed. Fitness levels of this population were similar to levels seen in trained female athletes. Iron status of our study population at the beginning of training was comparable to that of the general population. However, iron status dramatically changed over the training period, with two-thirds of the women iron deficient by the completion of the eight-week training course. The results of this study have shown that although no relationship was seen between iron status and performance, the basic training regimen appeared to negatively affect iron status and mood states. A similar finding on iron status reported for enlisted women suggests that basic training has a negative effect on iron status. This report is the first to document mood changes during basic training.

Nutritional status of women completing basic training remains a concern. Low nutrient intakes were reflected in significant decreases of serum markers of nutritional status by the completion of training. Women in the military continue to restrict food intake because of concerns about weight maintenance, leading to deficiencies in key nutrients. Although performance decrements were not seen in this group, long-term deficiencies may show different results. Chronic iron deficiency may also have an impact on numerous other health factors in individuals, such as injury and infection, ultimately affecting military readiness.

RECOMMENDATIONS

Regular monitoring of iron status in military women should be implemented. Iron status could readily be measured at the same time other blood work is done during initial entry processing for pregnancy and HIV status. For career women, this screening could be done at the time of their mandatory annual gynecological examination.

In addition to the monitoring of iron status, intervention and prevention programs should also be in place. Nutrition education programs that promote optimal iron intake as well as presentation of general nutrition principles can target women at risk for developing iron deficiency. For those women who are found to have iron deficiency or iron deficiency anemia, iron supplementation should be emphasized as an adjunct treatment with nutrition education.

Although we found no performance decrements short-term, these conditions may be manifested if impaired iron status continues past basic training for a sustained period of time. Further research should address the impact of chronic iron deficiency on measures of physical and cognitive performance for women in various military job specialties. The relationship between iron status and injury and illness should be examined. The role of iron status on performance measures and health status needs further investigation.

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APPENDICES

APPENDIX A

Demographic Data

DEMOGRAPHIC DATA

Description of women entering Army Officer Basic Training (n=57).

Characteristic		
Age (yr)	25.4 ± 4.2	
Height (cm)	163.6 ± 6.4	
Weight (kg)	60.5 ± 8.5	
Minutes run/wk	90.6 ± 71.0	
Exercise energy expended/wk (kcal)	2356 ± 1547	
Smoke (%) (n=3)	5.3	
Ethnicity (%): Caucasian	77.2	
Black	8.8	
Hispanic	8.8	
Other (Asian)	5.3	
Want to lose weight (%)	70.2	
Trying to lose weight (%)	45.6	
Lost weight last year (%)	49.1	
Birth control method (%)		
Birth control pills	49.1	
Contraceptive sponge	1.8	
Other	8.8	
None	40.4	
Part of country lived in longest (%)		
New England	5.3	
Middle Atlantic	24.6	
East North Central	10.5	
West North Central	12.3	
South Atlantic	12.3	
East South Central	3.5	

West South Central	5.3
Mountain	7.0
Pacific	12.3
Other	7.0
Marital status (%)	
Single	45.6
Married	42.1
Widowed/Divorced	12.3
Type of community lived in longest	
Central city	16.1
Suburbs	48.2
Rural	35.7

APPENDIX B

Energy Expenditure in Recreational Activities

Energy Expenditure in Reported Recreational and Sports Activities

Activity	Kcal/min	Activity	Kcal/min
Walking	4.5	Soccer	7.5
Jogging/Running	7.5	Rugby	7.0
Weight Training	6.0	Rollerblading	5.0
Stretching	4.0	Stairstepper	8.5
Aerobics	7.0	Softball	4.5
Calesthenics	7.0	LaCrosse	8.0
Swimming	7.0	Tennis	7.0
Basketball	7.5	Hockey	9.0
Racketball	7.0	Cross-country Ski	8.0
Horseback	7.0	Social Dancing	5.5
Biking	3.5		

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APPENDIX C

Major and Minor Food Groups

MAJOR AND MINOR FOOD GROUPS

Dairy

Fruits

Fluid Milk

Cream and Cream Substitute

Frozen Desserts

Cheese, Yogurt

Citrus Fruit Dried Fruit

Non-citrus

Fruit Juice and Nectars, Non-citrus

Meat

Beef

Pork Lamb Poultry

Organ and Prepared meats

Fish

Mixed Dishes/Meat and Starch Meat Extracts and Gravies Vegetables

Potatoes

Dark Green Vegetables Yellow Vegetables

Tomatoes

Other Vegetables

Eggs

Eggs, Alone Mixed Dishes

Egg Substitutes

Fats and Oils

Fats

Oils

Salad Dressings

Legumes

Legumes Nuts

Seeds

Carob Products

Sweets and Beverages

Sweets and Candies

Beverages, Non-alcoholic

Beverages, Alcoholic

Bread and Cereal

Grains and Flours

Breads

Quickbreads and Muffins Cakes, Pies, and Cookies Crackers and Salty Snacks

Pancakes and Waffles

Pasta and Cooked Grains Breakfast Cereals

Mixtures, Non-meat

Other

Condiments

Liquid Supplements

Vitamins

Minerals

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